Gastroprotective Effect of *Gnetum africanum* Ethanolic Extract on Acetic acid induced Peptic Ulcer on Adult Wistar Rats

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**ABSTRACT**

Peptic ulcer disease also known as stomach ulcer is a break in the lining of the stomach, first part of the small intestine or occasionally the lower esophagus. Twenty five Wistar rats of both sexes weighing between 180-250 grams were purchased, housed in metallic cages and maintained under standard environmental conditions at 27 ± 0.13°C at 12 hr light/dark cycle and relative humidity of 29 ± 0.22%. They were allowed to acclimatize for one week (7 days) and were fed on standard rodent diet and have free access to drinking water. However, they were divided into five groups of five rats per group, labeled A to E. Group A: served as Normal control and received normal feed and distilled water *ad libitum* Group B: induced with ulcer and left untreated) Groups C, D and E were induced with ulcer and treated with 20 mg/kg Omeprazole 200 mg/kg GALE extract, 400 mg/kg dose of GALE extract respectively. Hence, pretreatment with the extracts showed a significant (P<0.05) increase in pH value coupled with significant decrease in gastric volume when compared with the ulcerated untreated group. Also from Table 3 below, it was shown that normal control group (A) showed a 100% protection as no ulcer was induced into the group. Among the groups induced with ulcerations, the group that received 20 mg/kg omeprazole as standard drug had the best protection against ulceration followed by group D (400 mg/kg bw of GALE) while group C (200 mg/kg GALE) had a 50% protection against severe ulceration. GALE has been observed to possess phytonutrients with excellent antioxidant properties that play significant role in the management of ulceration or other toxicity related disorders. Treatment with *Gnetum africanum* leaf extract significantly reduced these parameters. In fact, the effect elicited by the extracts compared favorably with both normal control and the omeprazole treated groups.

**Keywords**: Peptic ulcer; Acetic acid; Proton pump inhibitors; *Gnetum africanum*

**INTRODUCTION**

Peptic Ulcer (PU) is a chronic development which is characterized by an imbalance between the factors (aggressive and defensive) that are harmful to the mucosa and its protection, finally occurrence of lesions on the lining of upper digestive tract [1] Peptic ulcer disease also known as stomach ulcer is a break in the lining of the stomach, first part of the small intestine or occasionally the lower esophagus. [2] An ulcer in the stomach is known as gastric while that in the first part of the intestine is known as duodenal ulcer [2]. This break or sore in the lining of the stomach or upper part of the small intestine has a diameter of at least 0.5 cm penetrating through the muscularis mucosa. Ulcers are primarily caused by an imbalance between some aggressive (acid pepsin secretion) and protective (mucous secretion, blood flow, cellular regeneration, prostaglandins, growth factors and integrity of mucosal barrier) endogenous factors in the gastro intestinal tract [3]. Several factors could also cause Peptic ulcer among which are: use of steroidal and non-steroidal anti-inflammatory but can lead to severe complications such as gastro intestinal bleeding, perforations, penetration of ulcer into adjacent organs and gastric outlet obstructions [4].
Pharmacological intervention utilizing histamine H₂-blockers, antacids and anticholinergic have not succeeded in conferring immunity from recurrence of disease or total restoration due to a number of limitations [5]. Gnetum africanum (African jointfr) is an edible plant widely used as food. It is an endangered liana normally found in humid tropical rainforest. It is usually found with other climbers on middle and understory trees frequently forming thickets, it can also be found in riveine forest in areas that are otherwise too dry for the species [6]. Gnetum africanum is adioeccious forest perennial liana up to 10m long with thick papery like leaves growing in groups of three but sometimes with longer branches somewhat thickened at the nodes [6]. In Africa, two different species of Gnetum are found: G. africanum and G. bucholzianum. They are distributed in the tropical rainforests from Nigeria through Cameroon, the Central African Republic, and Gabon and the Democratic Republic of the Congo to Angola [7]. In Nigeria, it is called ajage (Efik/ Ibibio); ukazi, ukazi (Gbogbo); yala (Ogoja); ajaabaje; ajakotale (Yoruba), in Cameroon it is known as eru, okos, mbumbu or fumbua and koko in Angola, Gabon and Central African Republic [8]. These two species are so similar that it is hard to distinguish them except by the shape of their leaves and the characteristics of the male reproductive organs. [9].

The leaves may grow approximately 8 cm long and at maturity the vine will produce small cone like reproductive structures. The seeds of the vine resemble a fleshy fruit sized 10-15 mm × 4-8 mm, and are red orange in color when fully ripe. It is traditionally a wild vine and is considered to be a wild vegetable [10]. The leaves can be chewed raw for the management of excessive urination by infantile diabetic patients as a traditional medicine [7]. Available literature also reported that both the leaves and the seeds have medicinal efficacy in the treatment of enlarged spleen, sore throats, reduction of pains during childbirth, antidotes to some forms of poison and snake bite. The seeds are specially used as fungicide for dressing fresh and septic wounds [11].

Hence this research work is aimed at assessment of the Gastroprotective properties of Gnetum africanum on acetic acid induced wistar rats. Different medications among which are antacids, antibiotics, proton pump inhibitors, other anti-secretory and cytoprotective agents are employed for pain relief healing of ulcers and for delaying recurrence. Clinical evaluation of most of these drugs has shown incidences of relapse, side effects and drug interactions.

In view of this, there is a need to search for drugs with greater or equal benefits but with reduced or minimal side effects among plants that can be used to treat peptic ulcer. Therefore, there is need to investigate the ulcer protective effect of Gnetum africanum in order to explore the possibilities of using it as a non-pharmacological means of preventing ulcer.

MATERIALS AND METHODS

Drugs and chemicals

Ethanol, acetic acid and other reagents that were used were purchased from Chemical reagent shops at form Ogbeo Main market Enugu and were of analytical grades, while the drugs were also purchased from Open heaven Pharmacy Ltd, Savage Crescent GRA Enugu.

Plant collection and authentication

The fresh leaves of Gnetum africanum were harvested from its natural habitat at Opi village in Nsukka LGA of Enugu State, Nigeria. It was identified and authenticated at the Department of Botany, University of Nigeria, Nsukka where a voucher specimen was deposited with voucher number UNH 17a.

Method of extraction of Gnetum africanum

The ethanolic extraction was done according to the method of [12]. The fresh leaves of Gnetum africanum were dried at room temperature (25°C ± 0.2°C) and pulverized with a mechanical grinder. (Binatone PLC USA). Approximately 112.7 g of the powdered plant was macerated with 800 mL of 96% ethanol for 24 hours at 4°C. The resulting mixture was filtered with Whatman No. 1 filter paper and evaporated to dryness in water bath at 40°C to get the concentrated aqueous crude extract with a yield of 3.72 g. The extract was stored in the refrigerator at 2°C until ready for use.

Phytochemical analysis of the ethanolic leaf extract of Gnetium africanium

The phytochemical screening of the extract of Gnetum africanum was carried following the method [13] at the department of Pharmacognosy of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka and the Phytochemical constituents were indicated as follows; Alkaloids, flavonoids, glycosides, saponin, tannins, steroids, terpenoid, carbohydrates and proteins at different concentrations.

Acute toxicity test of Gnetum africanum leaf extract

The acute toxicity test (LD₅₀) of the plant was analyzed using the newest method of acute toxicity by [2] stated below. Ten (10) matured wistar rats were procured, weighed and divided into three (3) experimental groups of four and three rats each.

Stage 1: Four animals were further divided into 3 groups of one rat each and Gnetum africanum leaf extract were given 50, 100, 200 and 300 mg/kg doses respectively orally and were monitored after 1 hour post administration for 10 mins at 2 hours interval for 24 hours. Since there was no sign of mortality or morbidity, the experiment proceeded to stage 2:

Stage 2: Three other rats were further divided into 3 groups and higher doses of 1000, 1500 and 2000 mg/kg body weight and were monitored as in stage 1. At the end of this stage, there were also no signs of morbidity or mortality. Then the experiment proceeded to stage 3.

Stage 3: Three other rats were procured and divided into three (3) groups of one animal each. Dosages of 3000, 4000 and 5000 mg/kg were administered and also monitored for signs of morbidity or mortality. At the end of stage 3, no death was recorded. Then the plant (Gnetum africanum) leaf extract was confirmed to have higher margin of safety even at dosages above 5000 mg/kg. Then the different dosages administered to each of
the animals in the experimental groups were selected for the study.

Animal housing

Twenty five wistar rats of both sexes weighing between 180-250 grams were purchased and housed in Laboratory Animal House, College of Medicine, Enugu State University of Science & Technology, Parklane Enugu, Nigeria. The animals were maintained under standard environmental conditions at 27 ± 0.13°C at 12 hr light/dark cycle and relative humidity of 29 ± 0.14%. They were allowed to acclimatize for one week (7 days) and were fed on standard rodent diet (Vital feeds limited Jos, Plateau State Nigeria) and have free access to drinking water.

Experimental design

Twenty five wistar were divided into five groups of five rats per group, labeled A to E.

Group A: Normal control and received normal feed and distilled water ad libitum

Group B: Induced with ulcer and left untreated

Group C: Induced with ulcer and treated with 20 mg/kg Omeprazole

Group D: Induced with ulcer and treated with 200 mg/kg GALE extract

Group E: Induced ulcer and treated with 400 mg/kg dose of GALE extract

Method of Induction of Ulcer

Acetic acid induced gastric ulceration

This method as described by Sanyal et al. [14]. The animals were fasted for 24 hours prior to the experiment. The animals were divided into five groups and all treatments were administered once daily for eight days with the gastric ulcer induced on the last day with 0.03 mL of 20% acetic acid. Group A received the vehicle only (10 mL/kg body weight). For all other groups an ulcer was induced on the last day of treatment, one hour after administering the compound and the ulcer scores were checked 8 hours post induction. After eight hours of post induction the rats were sacrificed and the stomach excised. The excised stomachs were fixed with 10% phosphate buffered solution for at least 15 minutes, and opened along the greater curvature to expose the gastric mucosal layer. Hemorrhagic lesions in the mucosal membrane of the glandular region were observed under a dissecting microscope and were manually scored. Scoring of ulcerations was patterned after assessment of rate of gastric acid secretion according to the method [14].

\[ \text{UI} = \frac{\text{TAML}}{\text{TMA}} \times 100 \]

Where TMA= Total mucosal area

TMLA=Total mucosal area of lesion [15]

Ul - Ulcer index

The protection percentage was calculated as below:

\[ \text{Prot} = \frac{(\text{UI of ulcerated control group} - \text{UI of treated group})}{(\text{UI of ulcerated control group})} \times 100 \]

Determination of gastric acid parameters

The volume of gastric acid secreted was determined in the supernatant by titration with 0.00025 N NaOH using Phenolphthalein as indicator. The pH (Pondus hydrogen) of the gastric acid secreted was determined using a pH meter with the procedure of hydrophobic barrier [16].

Method of collection of gastric acid and assessment of ulcer scores

After the induction of ulcer with the 0.03 mL of 20% acetic acid, the animals were sacrificed using 25% Urethane anaesthesia and the trachea cannulated using polyethylene tubing for aeration. The abdomen was exposed and the stomach isolated followed by an incision at the pyloric sphincter. After the incision, a little length of the polyethylene tubing was cut and inserted into the stomach through the incision made at the pyloric sphincter region and was ligated. A little quantity of normal saline was use to perfuse the stomach and the catheter set to collect the effluent at 10 mL/15 minutes. The volumes of the gastric acid collected were later titrated against the 0.00025 N NaOH solution. Then, the stomachs were harvested and opened through the greater curvature. They were rinsed under stream of normal saline, pinned flat on a cork board and were then observed with a magnifying lens (×10). Erosions formed on the glandular portion of the stomach were counted and given a score of 0-3 based on the diameter of the ulcer

0=no ulceration or almost formed

1=Vascular congestion

2=One or two lesions

3=Severe lesion

4=Very severe lesions

5=Mucosa full of lesion

Ethical clearance

Ethical clearance for this study was obtained from the Research Ethical Committee, College of Medicine, University of Nigeria Enugu Campus

Statistical analysis

Results were expressed as Mean ± standard error of mean (X ± SEM). For statistical analysis of data, multiple comparisons were carried out using one way analysis of variance (ANOVA) followed by a Tukey HSD test for post-hoc analysis. Statistical significance was acceptable at a level of p<0.05.

RESULTS

Table 1 below showed results of the qualitative and quantitative analysis of the Gnetum africanum leaf extract. It’s shown that the
plant possess many phytonutrients as stated in the table with highest concentration of alkaloid and carbohydrates with moderate concentration of saponin, glycosides and flavonoids. Phytonutrients like terpenoids, tannins and steroids were present in lower concentrations as enumerated in Table 1 below.

Table 1: Phytochemical analysis of Gnetum africanum leaf extract (GALE).

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Qualitative</th>
<th>Quantitative (mg/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+++</td>
<td>12.3</td>
</tr>
<tr>
<td>Saponin</td>
<td>++</td>
<td>7.2</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
<td>7.7</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>4.2</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>7.9</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>3.8</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+++</td>
<td>11.2</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Key: +++ =High concentration; ++ = moderate concentration; + = low concentration

Table 2 depicted the acute toxicity test of the plant which was done according to the method [17]. The plant (Gnetum africanum) showed a better therapeutic index or higher safety margin at doses greater than 5000 mg/kg.

Table 2: Acute toxicity test.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Recommended</th>
<th>Doses</th>
<th>mg/kg bw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>200</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>1500</td>
<td>2000</td>
</tr>
<tr>
<td>3</td>
<td>3000</td>
<td>4000</td>
<td>5000</td>
</tr>
<tr>
<td>LD50  &gt;5000 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The protective effect Gnetum africanum leaf on Acetic acid induced gastric ulceration is shown in Figure 1 below. Oral administration of 0.03 mL of 20% acetic acid caused a significant increase (P<0.05) in the degree of ulceration (ulcer index) of the rats. However, some significant protection/inhibition in the degree of ulceration was observed in the Gnetum africanum leaf extract. The extract at 400 mg/kg offered better protection than at 100 mg/kg dose of GALE but the standard anti ulcorogen (20 mg/kg omeprazole) had a better ulcer protection than the GALE. Meanwhile, ulcer scores/index was more pronounced in the ulcer untreated group.

Figure 1: Cytoprotective effect of ethanolic leaf extract of Gnetum africanum on Acetic acid induced gastric ulceration in wistar rats (n=5) on gastric acid secretion The bars with asterisk showed significant differences (P<0.05) compared to control group (A).

Figure 2: Protective effect of ethanolic leaf extract of Gnetum africanum on Acetic acid induced gastric ulceration in wistar rats (n=5) on ulcer scores/indices. The bars with asterisk showed significant differences (P<0.05) compared to control group (A).

Table 3: Percentage protection/inhibition and pH (Pondus hydrogen) exhibited by Gnetum africanum leaf extract on acetic acid induced ulceration in wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage protection (%)</th>
<th>pH (Pondus Hydrogen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100</td>
<td>6.5 ± 0.09</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>2.0 ± 0.12*</td>
</tr>
<tr>
<td>C</td>
<td>85 *β</td>
<td>5.7 ± 0.23* β</td>
</tr>
<tr>
<td>D</td>
<td>50 *β</td>
<td>3.5 ± 0.31* β</td>
</tr>
<tr>
<td>E</td>
<td>72 *β</td>
<td>4.0 ± 0.06* β</td>
</tr>
</tbody>
</table>

Keys: *P<0.05 showed a significant difference compared with group A (normal control) and B by<0.05 compared with group B.

However, Figure 2 shows the effect of ethanolic leaf extract of Gnetum africanum on gastric acid secretion on acetic acid induced ulceration in wistar rats. Administration of 20% acetic acid caused a significant (P<0.05) decrease in pH as seen in
Table 3 below with some corresponding increase in volume of gastric acid secreted as seen in the ulcer untreated group. Hence, pretreatment with the extracts showed a significant (P<0.05) increase in pH value coupled with significant decrease in gastric volume when compared with the ulcerated untreated group. Also from Table 3 below, it was shown that normal control group (A) showed a 100% protection as no ulcer was induced into the group. Among the groups induced with ulcerations, the group that received 20 mg/kg omeprazole as standard drug had the best protection against ulceration followed by group D (400 mg/kg GALE) while group D (200 mg/kg GALE) had a 50% protection against severe ulceration.

DISCUSSION

This study was aimed at investigating the cytoprotective effect of ethanolic leaf extract of *Gnetum africanaum* (GALE) on acetic acid induced gastric ulceration in wistar rats. It has been documented that the inhibitory action of prostaglandin synthesis coupled with free radical formation has been opined as critical biochemical events in the pathogenesis of gastric ulceration. [18-20]. GALE has been observed to possess phytonutrients with excellent antioxidant properties that play significant role in the management of ulceration or other toxicity related disorders. Interestingly, many research have reported the presence of these phytonutrients which enable them to promote good health [21,22]. The study also investigated the acute toxicity test of the plant (GALE) and discovered that it has a very high safety margin and it’s properly safe for human consumption even at dose above 500 mg/kg. The safety of the plant might be attributed to its high concentration of some of the phytonutrients such as carbohydrates, alkaloid etc. [23]. Meanwhile the biochemical analysis of the plant for gastric secretion (PH and volume) was employed to ascertain its status following the administration of some pharmacological agents [24]. Hence, the pH gives idea of the level of acidity and level of gastric secretion. Low pH values is a manifestation of the decreased hydrogen ion concentration as this has been linked to the pathogenesis of ulcer and gastric damage in experimental animal. Inas et al. has also attributed the gastrointestinal injury as to eroded mucin content. The erosion is facilitated by onslaughts of both internal and external aggressive agents on mucosal epithelia.

Hitherto, the significant increase in ulcer index and gastric volume following oral administration of acetic acid in the ulcerated rats may be attributed to either free radical formation or inhibition of prostaglandin synthesis. Decreased prostaglandin level has been attributed to impaired gastroprotection and increased gastric acid secretion which are important events in the etiology of mucosal ulceration and this research agreed with the work [12].

Finally treatment with *Gnetum africanaum* leaf extract significantly reduced these parameters. In fact, the effect elicited by the extracts compared favorably with both normal control and the omeprazole treated groups respectively thus is suggestive of possible protective effect.

CONCLUSION

The attenuation of the gastric affronts of indomethacin by administration of ethanolic extracts of *Gnetum africanaum* at 200 and 400 mg /kg body weight regimen is a clear indicative of their excellent gastroprotective potential in rats. Meanwhile, further research is important in investigating the exact mechanism of the protective effect or antiulcerogen effect of *Gnetum africanaum* leaf extract and also harnesses its possible synergistic efficacy against ulcer. The researcher also recommends the adequate consumption of this plant (*Gnetum africanaum*) in our daily diets.

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CONFLICT OF INTEREST

None to declare

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